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INACTIVATION OF *GIARDIA* CYSTS BY IODINE WITH SPECIAL REFERENCE TO GLOBALINE: A REVIEW

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This review of the literature indicates that iodine is not completely effective in destroying Giardia especially in cold (3 to 15°C) water. Cysticidal efficacy of iodine at a given concentration and temperature depends on contact time. The iodine tablet, Globaline, used by U.S. Military Forces since 1952, required 60 minutes at 15°C, 30 minutes at 25°C and 25 minutes at 45°C for 100% destruction of Giardia cysts at residual iodine concentration of 10, 8, and 6 mg per liter, respectively. The time required at temperatures below 15°C for Globaline tablets to destroy Giardia is not presently known. Based on the information presented in this review, two changes for Globaline are recommended: (1) reformulate Globaline tablets with an alkaline buffer and (2) increase the treatment time for one liter of water from 35 minutes to 60 minutes.

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PREFACE

Protocols for treatment of canteen water with iodine tablets, called Globaline(R), are presently based on obtaining the destruction of a resistant waterborne protozoan known as Giardia lamblia. Current procedures for treatment of field water with Globaline, including contact time are presented in Military Specification MIL-W-283H (11) and field manuals such as FM 21-10 (5).

This review summarizes current knowledge about the efficacy of iodine for destruction of Giardia lamblia with special reference to Globaline iodine tablets in cold water. In addition, this report updates and reaffirms information on the use of iodine tablets for the purification of field water and suggests revisions to relevant specifications and manuals. The text can serve as a basis for instructing soldiers on the proper treatment of field water, if necessary. Research investigators may use this report as a ready reference and guide for ongoing research, such as the development of other water purification tablets, development of compatible beverage powders or for the identification of additional areas for research.

The literature reviews include government contract reports, special studies and journal articles dating from 1945 through 1990. Results from the literature pertaining to iodine, particularly Globaline, are summarized in the following tables and appropriate references are listed.

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The citation of trade names in this report does not constitute an official endorsement or approval of the use of a product.

INACTIVATION OF <u>GIARDIA</u> CYSTS BY IODINE WITH SPECIAL REFERENCE TO GLOBALINE : A REVIEW

INTRODUCTION

The Globaline iodine tablet (11) was developed for the U.S. Armed Forces during World War II (6) to replace the Halazone Chlorine tablet, which had been used since World War I for disinfecting canteen water (14). A research and development program was sponsored by the Army Quartermaster General and conducted by Harvard University investigators (6). To overcome the inherent limitations of Halazone which affected its efficacy, the Harvard investigators proposed the following criteria for the ideal water purification tablet for field use (6, 9, 14). The tablet should:

1. Be effective as a single tablet and be simple to use.
2. Dissolve and liberate its active ingredient rapidly.
3. Destroy all waterborne pathogens and be effective in all kinds of water.
4. Provide treated water that is acceptable and nontoxic.
5. Be stable during storage and actual use.

An intensive search was undertaken by the Harvard investigators (6) for a compound that would satisfy these criteria. After screening more than 57 iodine - and chlorine - releasing compounds, Globaline was selected as the tablet that best satisfied military requirements (14). It was first issued to American soldiers in 1952.

Globaline (MlL-W-283H) is composed of tetraglycine hydroperiodide[(18) active ingredient], sodium acid pyrophosphate and talc (9, 11, 14). One tablet dissolves in a liter of water to yield from 7.6- 9.0 ppm titratable iodine(11).

To purify field water the soldier is instructed (5, 11) to add two Globaline tablets to a full canteen of water (1 quart or 1.1 liter), wait 5 minutes for the tablets to dissolve, shake the water for an indeterminate time period and wait an additional 30 minutes. No reference is made to water temperature. Contradictory instructions on labels of bottled Globaline tablets manufactured as late as January, 1989, instructed the soldier to wait for only 20 minutes after shaking.

It is generally known that the germicidal and cysticidal activity of disinfectants is slowed by low temperatures (3, 4, 6, 7, 8, 9, 13, 15, 16, 17, 19). Therefore, there is great concern that two Globaline tablets may not effectively purify cold water (5°C) by destroying <u>Giardia</u> cysts within the recommended 20 to 35 minutes. <u>Giardia</u> represents a known hazard in cold water streams, such as those found in the United States, from New Hampshire to Washington (7).

The use of chlorine and iodine in water treatment is universally accepted and the effectiveness of both compounds in destroying pathogenic bacteria in public water supplies is well recognized. Accordingly, this report does not address the voluminous literature concerning bacteria. However, a review of the literature revealed that there is a paucity of information regarding the efficacy of iodine against <u>Giardia</u> cysts and even less information on the efficacy of Globaline, at temperatures below 15°C. Guidelines do not exist for

chemical treatment of public drinking water to destroy Giardia, because little is known of the effect of halogens on Giardia cyst viability. Furthermore, guidelines used for Globaline were originally based on killing Entamoeba histolytica cysts (3, 6), not Giardia. Until 1980, none of the chemical methods available for disinfecting drinking water had ever been tested for destruction of Giardia cysts (7). Because protozoan cysts are the most resistant of the waterborne pathogens to disinfectants, treatments effective against them will also be effective against bacteria and other microorganisms.

It was recognized in 1986 (personal communication, Morris Rogers, Natick, memorandum for the record, subject: Giardia lamblia in military water supplies, 25 March 1986) that Globaline (iodine) had serious limitations in cold water regarding the destruction of Giardia. This conclusion was based on work presented by Jarroll (7).

Therefore, this review addresses the specific questions concerning the ability of Globaline to destroy Giardia cysts in cold water within the recommended time period.

CYSTICIDAL EFFICACY OF DIFFERENT FORMS OF IODINE

Influence of contact time and form of iodine on destruction of Giardia

Table 1 summarizes comparative data for the various forms of iodine, which are presented in more detail in Tables 2 thru 5. Although the test waters differed, the minimal contact times required for comparable residuals of various forms of iodine to destroy Giardia at various water temperatures are presented. Only Globaline achieved 100% destruction of Giardia, but it required 60 minutes contact time at 15°C and at least 10 mg/L of residual iodine (15). At 25°C only 30 minutes was required even though the residual iodine was less. The lowest temperature studied with Globaline was 15°C. Less residual iodine was required as temperature increased. Only 99% kill times were determined in the studies with elemental iodine (16,17). Contact time and the residual elemental iodine required decreased as temperature increased. Saturated iodine (8) and tincture of iodine (7) were more effective at 20°C than at 3°C (see tables 3 and 5), but did not completely kill Giardia even after 30 to 40 minutes. Iodine residuals were lower than they were in studies with elemental iodine and Globaline. In the studies with all four forms of iodine, it is clear that longer contact times and higher iodine residuals are required in cold water (< 25°C) to completely destroy Giardia. Test waters included hard organic water (HOW: see appendix), deionized, distilled, tap, and river water.

Globaline

Table 2 presents the efficacy of Globaline for destruction of Giardia and E. histolytica cysts at temperatures ranging from 3 to 45°C (6, 7, 9, 10, 13, 15). Cyst densities were 1000 Giardia per mL (6) and 60 E. histolytica per mL (9,13). It is evident that 20 or 30 minutes was not sufficient time to destroy Giardia at 3 to 15°C even with two tablets in natural river water or HOW (7,15). A 100% mortality was achieved in HOW only after 60 minutes at 15°C, 30 minutes at 25°C and 25 minutes at 45°C (15). Residual iodine levels were 10 ppm, 8 ppm and 5.7 ppm, respectively. The HOW was a worst case water

that was made up to have a high iodine demand, high pH (pH 9), and high total dissolved solids (>1500 mg/L). Cysticidal activity was most dependent on contact time since less residual iodine was required as the temperature of the water was increased (7). Chang (4) showed that cysticidal action of halogens decreased as temperature decreased. The contact time required to destroy Giardia at temperatures between 3 and 15°C and between 15 and 25°C is not known. Activity of Globaline appeared to be independent of pH over a range of pH 4 to 8. Giardia was more resistant to Globaline than E. histolytica.

Saturated Iodine

Table 3 shows that saturated iodine did not completely destroy Giardia cysts (1000/mL) at 3°C even after 40 minutes contact time whether the water was clear or cloudy (8). As indicated by the data, efficacy could be improved by increasing the contact time of the residual iodine. At 20°C in both clear and cloudy water, 99.8% of the Giardia cysts were destroyed after only 15 minutes even though residual iodine levels were lower than at 3°C (7, 8). As with Globaline, pH appeared not to be a factor at the ranges shown.

Elemental Iodine

Studies with elemental (aqueous) iodine are presented in Table 4. Higher concentrations of elemental iodine were required to destroy Giardia cysts (10,000/mL) at 5°C than at 15 to 25°C (16, 17) in distilled water. The complete destruction of E. histolytica cysts (30 to 60/mL) in sewage diluted tenfold in Cambridge tap water (SCTW) required twice as much contact time (20 minutes) at 5°C than at 23°C, at the same iodine concentration (2). At low temperatures (0 to 5°C), a contact time of 10 minutes was not sufficient(3). In studies with E. histolytica, the cysticidal activity of iodine was independent of pH in the range between pH 3 to 7 (3). High germicidal activity was maintained over the pH range of 3 to 8 in the presence of a variety of water contaminants (3, 19). Having less dependence on pH was considered to be one of the advantages of using iodine to disinfect water. Lower residuals were required to destroy Giardia as the contact time increased (16, 17).

Tincture of Iodine

Table 5 shows that inactivation of Giardia cysts (1000/mL) by 2% tincture of iodine was more effective at 20°C than at 3°C in clear (deionized) water (7, 10). The failure of tincture of iodine at 3°C in clear water may have been due to insufficient contact time, since lower residuals inactivated Giardia cysts at 20°C (7, 10). Complete inactivation of Giardia was not achieved at the contact time and residual iodine levels studied.

CHEMICAL AND PHYSICAL PROPERTIES OF GLOBALINE

Effect of pH and titratable Iodine

When elemental iodine(I_2) is dissolved in water, the I_2 may remain unchanged or it may hydrolyze into five different species depending on pH and the initial concentration of titratable iodine (1,4,9,19). Of the five species produced during hydrolysis of I_2, only I_2 and hypoiodous acid (HOI) are

important for disinfection. Both are known to be powerful disinfectants (4, 9, 13, 19). The I_2 is more cysticidal (4,9) and sporicidal (19) than HOI, but the viricidal efficacy of HOI is 44 times greater than I_2 (4, 9). The sporicidal action (99% killing time of <u>Bacillus</u> <u>metiens</u> spores) was not affected at pH values between 6 and 7.5, where sufficient I_2 was present, but it decreased at pH 8 and above as the HOI fraction increased (19). Therefore to be effective as a broad spectrum disinfectant, both I_2 and HOI should be present in solution (4, 9, 13).

Table 6 shows that an insignificant amount of I_2 is hydrolyzed below pH 6 (1, 4, 9). All the titratable iodine exists as I_2 below pH 6 when the total titratable iodine is 0.5 ppm or greater. The same is true below pH 7.0 when the total titratable iodine is 5 ppm or greater. At pH 7.0, the amount of I_2 hydrolyzed to HOI ranged from 48% at 0.5 ppm to 10% at 5.0 ppm titratable I_2. The percent I_2 and HOI present were about equal at pH 7.0 in a dilute iodine solution (0.5 ppm) and also at pH 8.0, at 5.0 ppm titratable I_2. The decomposition of HOI to form iodate is slow and depends on the buffering system and the time of standing (4). Chang (3) demonstrated that the concentration of I_2 required for effective disinfection of <u>E</u>. <u>histolytica</u> cysts was about 5 ppm at 23°C for 10 minutes in clear water. Therefore, to allow for the iodine demands of natural waters, it was concluded that a suitable dose of iodine for emergency disinfection was about 8 ppm (3, 4). Water with I_2 demands greater than 4 ppm required twice the dose of I_2 and waters near 0°C required an increased treatment time of 20 minutes (3, 4). Consequently, Globaline tablets were formulated to provide 8 ppm of titratable I_2 per liter of water (6) which gave a sufficient I_2 residual to destroy 30 cysts of <u>E</u>. <u>histolytica</u> within 10 minutes in most natural waters at 23°C (3, 4).

The effectiveness of Globaline tablets for disinfecting cysts of <u>E</u>. <u>histolytica</u> was presented by O'Connor (13) and Kapoor (9). As shown above in Table 2, one Globaline tablet at 10°C lowered the initial pH of tap water to pH 6.6, which left a residual of 6.9 ppm of I_2. Since Chang (4) showed that this resulted in only about 5% HOI (Table 6), the final HOI concentration would be about 0.35 ppm (6.9 x 0.05). This small amount of HOI may not be sufficient to be viricidal. Therefore, the high concentration of titratable I_2 and the use of acidic buffer result in a high cysticidal but low viricidal efficiency (9, 13).

<u>Thermal Stability of Globaline</u>

Military criteria for water purification tablets require that they be chemically stable during storage for five years as packaged. The tablets must also withstand extremes of temperature encounted in different parts of the world and in storage warehouses.

The stabilty of Globaline tablets at ambient temperatures over a six - year period stored in the original, wax - sealed brown glass bottle was determined by measuring residual iodine levels for tablets produced in 1983 and in 1989, as shown in Table 7 (Powers, E. M., 1989, unpublished). Each of ten tablets was dissolved in one liter of halogen - free deionized water. The average tablet produced between 7.1 to 8.23 mg/L of residual iodine.

Therefore, two tablets added to a liter of water will provide sufficient iodine residuals to destroy Giardia at the contact times indicated in Table 1. Globaline tablets exhibited excellent storage stability and product reliability since residual iodine levels measured for tablets in 1983 were approximately the same as tablets produced in 1989.

Table 8 shows the thermal stability of Globaline tablets during accelerated storage studies at 60°C (140°F) and ambient humidity. Tablets produced in 1989 were exposed in the original 20 x 50 mm screw - capped, sealed bottles, in which they are packaged 50 tablets to a bottle, as well as exposed to the air in open petri dishes (Powers, E.M., unpublished). Two tablets from each container were removed periodically over a 30 day period. Each tablet was dissolved in one liter of deionized water and after a 30 minute waiting period the total residual iodine was titrated amperometrically (Wallace and Tiernan Titrator). Results from the Harvard Study (6) in 1945, in which tablets were exposed to the air on watch glasses, are presented for comparison. Most of the iodine was retained over the 30 - day period by tablets stored in tightly capped bottles. However, when exposed to the air, tablets lost iodine rapidly after 7 days and 66% had dissipated after 25 days. Results in 1945 were similar (6).

In similar studies with powdered Globaline exposed to 60°C on open watch glasses, the Harvard Group (6) reported a 50% loss of iodine after 49 days and a 92% loss after 103 days. Therefore, bottles of Globaline tablets must be tightly sealed after removal of each tablet. Under these conditions, deterioration of Globaline will be slow even at elevated temperatures.

Effect of Temperature on Dissolution Time

Rapid solubility is an important criterion for acceptability of Globaline tablets. Field trials with soldiers indicated that they became impatient with agents that required a waiting period of more than 10 minutes (6, 9, 13). Therefore, since dissolving time must be added to the time required for germicidal activity to obtain a total treatment time (12), the germicide must be dispersed quickly and uniformly to be most effective and to achieve adequate treatment of water. Globaline has a solubility of 380 grams per liter of distilled water (5, 9, 13, 18).

In simulated field studies, a Globaline tablet was placed in a liter of tap water at 23°C (6, 9, 13). The flask containing the water was inverted end-over-end continuously until the tablet was dissolved. Dissolution times, observed visually, were as follows:

Temperature °C	Dissolution Time (seconds)
10	114
20	72
23	46
30	48
40	30

To be considered satisfactory for field use tablets must dissolve within one minute at 23°C (6, 12). As shown above, Globaline dissolved within 46 seconds at 23°C. Dissolution time becomes shorter as temperature is increased. It was also shown that storage of Globaline at 60°C and room humidity did not affect the dissolving properties (6).

<u>Resistance of Globaline to Humidity</u>

To determine the stability of Globaline in humid atmospheres, the Harvard Group conducted experiments at humidities encountered in jungle warfare (6). The Globaline tablets were placed on flat dishes in desiccator jars controlled at humidities of 100%, 79%, and 55%. Table 9 presents the results of these studies. The tablets were hygroscopic and unstable at 100% humidity, losing 63% of their iodine in only two days. They were completely dissolved after 4 days. At 79% humidity, tablets lost more than half of their iodine after only six days. Globaline tablets were much more stable at 55% humidity retaining 54.2% of the iodine after 111 days. In another study (6) of simulated field conditions, bottles of Globaline were placed in a control room held at 80 to 90% humidity and 26.7°C(80°F). During the day, bottles were opened for a minute and a tablet was withdrawn. None of the tablets showed an appreciable loss of active iodine after three weeks, and there was little variation between the bottles with or without cotton plugs. When compared to four other halogen compounds, Globaline was shown to be the most stable at normal (32%) relative humidity.

DISCUSSION

This review of the literature indicates that most procedures with Globaline and other iodine solutions did not achieve adequate or complete destruction of <u>Giardia</u> cysts, in cold water (7, 8, 10, 16, 17). The failure of these methods to inactivate <u>Giardia</u> appeared to be due to an insufficient contact time. It was shown that cysticidal and sporicidal action of halogens decreases as the temperature decreases (4, 19). Wyss and Strandskov (19), for example, showed that lowering the temperature 10°C increased the killing time of <u>Bacillus</u> <u>metiens</u> spores fourfold. Therefore, temperature must be considered when iodine is used for disinfection of drinking water.

Because disinfection follows a first - order chemical reaction (2, 3, 4, 16), there is an inverse relationship between concentration of iodine and contact time with microorganisms. This means that low concentrations of iodine will require longer periods of time to kill <u>Giardia</u> and other microorganisms. The disinfection reaction is also slowed by cold temperatures.

The development of the Globaline iodine tablet during World War II (6) was a major breakthrough in water purification and provided the soldier with an effective method of producing potable water under emergency conditions. It has satisfied most of the requirements established for a water purification tablet and was a much more efficient disinfectant than the Halazone tablet which it replaced. Although the development of Globaline by Harvard scientists (6) was a tremendous effort, they operated under some basic assumptions that are no longer upheld:

The first assumption was that cysts of E. histolytica were the most disinfectant resistant waterborne pathogen and should be used as the test organism. It is now known that Giardia cysts are much more resistant to disinfectants, including iodine, as shown in Tables 2 and 4. Consequently, guidelines presently used for Globaline (two tablets per liter) are based on the destruction of Giardia. The second assumption was that molecular iodine alone was the germicidal species and not products produced by hydrolysis of iodine. We know now that while I_2 is a very good cysticidal agent, it is not as viricidal as HOI (9, 13). Therefore, both I_2 and HOI must be present in solution to be cysticidal as well as viricidal. The concentration of both species is dependant on pH and total residual iodine.

The Harvard scientists (6) assumed that the destruction of viruses by disinfectants was of the same order of magnitude as that of most pathogenic nonsporulating bacteria. Thus, as suggested by O'Connor (13) and Kapoor (9), it would be useful to evaluate the viricidal power of Globaline tablets. Because sodium acid pyrophosphate buffer present in Globaline (11) lowers the pH, the amount of HOI liberated may not be sufficient to be viricidal (9, 13). Kapoor (9) suggested that Globaline could be improved by substituting an alkaline buffer (pH 8), which would yield almost equal percentages of I_2 and HOI (Table 6).

The cysticidal efficacy of Globaline at temperatures below 15°C has not been sufficiently studied and it is uncertain at the present time. Therefore, there is great concern that Globaline tablets may not effectively disinfect some waters encountered by American soldiers within the recomended 35 minute treatment time. Studies are now underway under contract from the U.S. Army Natick R D and E Center, that will provide efficacy data at 5°C.

Based on the information presented in this review, two changes for Globaline are recommended: (1) reformulate Globaline tablets with an alkaline buffer and (2) increase the treatment time for one liter of water from 35 minutes to 60 minutes.

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APPENDIX

Hard Organic Water (HOW) Suspending Medium.

The final composition of the hard organic water was as follows:

$NaHCO_3$	0.0042 M
$KHCO_3$	0.0024 M
$CaCl_2$	0.0019 M
$MgCl_2$	0.0011 M
Tea infusion	0.15%

The sodium bicarbonate and potassium carbonate were made up as 100 x solutions (0.42 and 0.24 M, respectively). The calcium and magnesium chloride solution were made up as 1,000 x solution (1.9 and 1.1 M, respectively). One commercial tea bag was boiled in one liter of water for 30 minutes. All solutions were autoclaved and diluted before use.

Table 1. Summary[a] of comparative contact times and concentrations for different forms of iodine to destroy *Giardia* cysts.

Iodine	Temp. °C	Water	pH	Residual iodine (mg/L)	Contact time (minutes)	Percent mortality	Reference
Globaline (2 tablets)	15	HOW[b]	9	10	60	100	15, Table 2
	25	HOW	9	8	30	100	15, Table 2
	45	HOW	9	5.7	25	100	15, Table 2
Elemental	5	Distilled	7	22.0	24	>99	16,17, Table 4
	15	Distilled	7	11.4	21	>99	16,17, Table 4
	25	Distilled	7	11.2	6.3	>99	16,17, Table 4
Saturated	20	River	7	3.01	15-40	>99.8	7,8,10,Table 3
	20	Deionized	7.1	3.3	15-40	>99.8	7,8,10,Table 3
Tincture(2%)	3	River	7.4	4.1	30	>99.8	7,10,Table 5
	20	River	7.1	3	30	>99.8	7,10,Table 5

[a]Data taken from following Tables 2 through 6.
[b]Hard organic water plus tea infusion (see Appendix for composition)

Table 2. Destruction of protozoa cysts by Globaline[a] at various temperatures.

Temp °C	Water	No. of tablets per liter	Contact time (minutes)	Residual I_2 (mg/L)	Final pH	Percent mortality	Reference
			Giardia (1000/mL)				
3	Clear[b]	1	20	6.8	4.6	97.5	7,10
3	Cloudy[c]	2	20	9.88	5.8	>99.8	7,10
15	HOW[d]	2	30	10.4	8.	99.34	15
15	HOW	2	45	8.9	8.1	99.98	15
15	HOW	2	60	10.0	8.1	100	15
20	Clear	1	20	5.44	5.1	>99.8	7,10
20	Cloudy	2	20	9.47	5.7	>99.8	7,10
25	HOW	2	20	8.73	8.1	99.94	15
25	HOW	2	30	8.03	8.1	100	15
45	HOW	2	5	7.24	8.1	99.6	15
45	HOW	2	10	4.18	8.1	99.7	15
45	HOW	2	15	5.2	8.1	99.8	15
45	HOW	2	20	5.6	8.3	99.98	15
45	HOW	2	25	5.7	8.4	100	15
			Entamoeba histolytica (60/mL)				
3	Tap	1	25	7.5	6.5	100	6,9,13
10	Tap	1	15	6.9	6.6	100	6,9,13
23	Tap	1	10	7.5	6.6	100	6,9,13
23	Tea Infusion	2	5	8.7	6.4	100	6,9,13
28	Tap	1	5	7.5	6.6	100	6,9,13

[a]Tetraglycine hydroperiodide
[b]Clear - deionized, halogen free
[c]Cloudy - Willamette River water, Portland, Oregon
[d]Hard organic water plus tea infusion

Table 3. Destruction of *Giardia* cysts (1000/mL) by saturated iodine in clear and cloudy water at 3°C and 20°C.

Temp. °C	Water condition[a]	Stock iodine solution (mL)	Contact time (minutes)	Residual I_2 (mg/L)	Final pH	Percent mortality	Reference
3	clear	26	15	6.87	7.1	56.5	8
			20	7.09	5.7	96.5	7,10
			30	6.87	7.1	93.3	8
			40	6.87	7.1	95.9	8
	cloudy	26	15	4.58	7.3	37.2	8
			20	3.22	7.1	77.3	7,10
			30	4.58	7.3	57.5	8
			40	4.58	7.3	81.5	8
20	clear	13	15	3.15	6.0	>99.8	7,10
			15	3.29	7.1	>99.85	8
			30	3.29	7.1	>99.85	8
			40	3.29	7.1	>99.85	8
	cloudy	13	15	3.01	7.3	>99.85	8
			20	3.01	7.0	>99.84	7,10
			30	3.01	7.3	99.85	8
			40	3.01	7.3	>99.85	8

[a]Clear water was halogen free deionized water; cloudy water was from the Willamette River, Portland, Oregon.

Table 4. Destruction of protozoa cysts by elemental iodine[a]

Temp °C	Water	pH	Contact time (minutes)	Residual I_2 (mg/L)	Protozoa	Percent mortality	Reference
5	DW[b]	7	4.7	60.0	G[c]	99	
16,17							
	DW	7	10.4	36.4	G	99	16,17
	DW	7	23.6	22.2	G	99	16,17
	SCTW[d]	3-7	20	8.0	E[e]	100	3
6	SCTW	8	5	14.1	E	100	4
15	DW	7	13.0	15.2	G	99	16,17
	DW	7	21.2	11.4	G	99	16,17
23	SCTW	3-7	10	8.0	E	100	3
25	DW	8	5	7.1	E	100	4
	DW	5	3.8	9.2	G	99	16,17
	DW	5	10.0	5.9	G	99	16,17
	DW	5	38.0	3.6	G	99	16,17
	DW	7	3.2	15.4	G	99	16,17
	DW	7	6.3	11.2	G	99	16,17
	DW	7	17.8	6.2	G	99	16,17
	DW	9	2.0	37.0	G	99	16,17
	DW	9	10.0	23.0	G	99	16,17
	DW	9	11.0	19.0	G	99	16,17
	DW	9	44.6	10.0	G	99	16,17

[a]Iodine dissolved in distilled water
[b]DW - Distilled water
[c]G - Giardia cysts, 10,000/mL
[d]SCTW - 10% sewage in Cambridge tap water
[e]E - Entamoeba histolytica cysts, 30 to 60/mL

Table 5. Destruction of *Giardia* cysts (1000/mL) by two percent tincture of iodine in clear and cloudy water at 3 and 20°C

Temp. °C	Water condition	2% Tincture of iodine (mL)	Contact time (minutes)	Residual I_2 (mg/L)	Final pH	Percent mortality	Ref.
3	Clear[a]	0.25	30	3.37	5.8	74.6	7,10
	Cloudy[b]	0.5	30	4.08	7.4	99.8	7,10
20	Clear	0.25	30	3.22	5.8	99.8	7,10
	Cloudy	0.5	30	2.79	7.1	99.8	7,10

[a]Clear water was deionized, halogen free water.
[b]Cloudy water was obtained from the Willamette River at Portland, Oregon.

Table 6. Effect of pH and concentration on hydrolysis of iodine in aqueous solution

	Titratable iodine							
	0.5 ppm[a]		1 ppm[b]		3 ppm[b]		5 ppm[c]	
pH	I_2	HOI	I_2	HOI	I_2	HOI	I_2	HOI
5	99	1	99	1	100	0	100	0
6	90	10	95	5	98	2	98	2
7	52	48	67	33	82	18	90	10
8	12	88	20	80	40	60	50	50

[a]Adapted from reference 1 (Black, 1965)
[b]Adapted from reference 4 (Chang, 1958)
[c]Adapted from reference 9 (Kapoor, 1969)

Table 7. Residual iodine released by Globaline in water at 25°C[1]

Year of manufacture	No. tablets	Residual I_2 (mg/L)[2]	
		Range	Average[3]
1983	10	7.16 - 9.31	8.23
1989	10	5.01 - 7.88	7.1
1989	10	5.01 - 7.88	7.1

[1]Powers, E.M. 1989. U.S. Army Natick RD&E Center, Natick, MA 01760-5020.

[2]One tablet dissolved in 1 liter of iodine demand-free deionized water for 30 minutes.

[3]Average of 10 tablets.

Table 8. Thermal stability of Globaline tablets stored at 60°C (140°F)

Days at 60°C	Average percent loss of residual iodine		
	Screw capped bottles[a]	Open[a] Petri dish	Open watch glass[b]
1	15	0.5	5
2	17	8.5	2
3	15	19	9
7	22	29	37
9	22	—	42
25	27	66	—
30	27	66	—

[a] Powers E.M, 1989
[b] Adapted from the Harvard study, 1945 (6)

Table 9. Loss of iodine from Globaline tablets in different humidities at room temperature.[a]

	Percentage of iodine lost		
Time in days	100% humidity	79% humidity	55% humidity
0	0	0	0
1	32.3	12.3	11.1
2	63	ND	13.9
3	84.6	9.6	ND
4	b	31.5	22.2
5	ND[c]	38.4	19.4
6	ND	53.4	19.4
7	ND	ND	20.8
100	ND	ND	54.1
111	ND	ND	54.2

[a]Adapted from reference 6
[b]Tablet dissolved after 4 days
[c]Not done